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# Community-acquired infectious diarrhea among children under five years in Dakar, Senegal

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Running head

*J-M Sire et al.*

Infectious infantile diarrhoea in Dakar

**Background:** In sub-Saharan Africa, infectious diarrhoea is a major cause of childhood morbidity and mortality. A cross-sectional study was undertaken to document the pathogens potentially involved in community-acquired childhood diarrhoea in Dakar, the capital of Senegal.

**Methods:** Between September 2007 and March 2008, 176 children aged 1 month to 5 years were recruited consecutively from a primary health care institution in an urban area. Clinical data were recorded and stool samples were collected. Bacterial pathogens were identified using conventional methods and/or PCR assays. Rotaviruses and adenoviruses were detected by a rapid immunochromatographic test. Intestinal parasites were diagnosed by microscopy.

**Results:** Rotavirus was the most common enteric pathogen, detected in 27% of patients, followed by Shigella (12%), diarrhoeagenic *Escherichia coli* (8%), enteric adenovirus (8%), Salmonella (4%), *Campylobacter jejuni* (3%) and *Plesiomonas shigelloides* (2%). Mixed bacterial/viral infections were detected in 6% of cases. Parasites, mostly protozoa, were detected in 14% of children. Using *ipaH* PCR, 30% of samples were positive for Shigella/entero-invasive *E. coli*. Detection of rotavirus was more frequently associated with younger age groups (<24 months), whereas bacterial diarrhoea was isolated more often in children over 1 year of age. Detection of bacterial pathogens was significantly associated with malnutrition. Antibiotics were prescribed in 77% of children who attended to the consultation. No pathogen was found in 36% of them, whereas a virus was detected without any other associated bacterial or parasitic pathogen in 23%.

**Conclusion:** In developing countries, there is a need to develop reliable, easy-to-use, inexpensive rapid diagnostic tests to guide the management of diarrhoea in infants and children and thereby prevent over-use of antimicrobial agents.

**Keywords:** Infectious diseases; Community-acquired diarrhoea; Children; Dakar; Senegal

## Introduction

Infectious diarrhoea remains a major cause of morbidity and mortality in developing countries, particularly in children. It has been estimated that in 2010 it accounted for 0.801 million deaths worldwide in children under 5 years. Between 2000 and 2010, deaths from childhood diarrhoea decreased by 0.309 million.<sup>1</sup> However, the morbidity has not declined, especially in Africa, probably because risk factors (poor hygiene, lack of access to potable water, insufficient promotion of breastfeeding, malnutrition) still prevail.<sup>2</sup> The continued growth of many major African cities with resulting overcrowding may also contribute to outbreaks of diarrhoea to which children are particularly vulnerable. Data are needed in these urban areas to establish the local epidemiology and implement appropriate preventive measures. We report a cross-sectional study undertaken in a dispensary in central Dakar in order to detect the pathogens and clinical characteristics of community-acquired infantile diarrhoea.

## **Subjects and Methods**

### *Patients*

Between September 2007 and March 2008, children aged 1 month to 5 years with acute diarrhoea were enrolled consecutively from the Saint-Martin dispensary, a Catholic mission-run primary health care organisation for maternal/child health in Rebeuss, a poor and densely populated district of central Dakar. Children enrolled had had at least three stools (aqueous, slimy/mucous or bloody) per day, symptoms for <7 days, and had not received any antibiotic treatment during the previous 7 days. Clinical examination was undertaken after taking a history from the parents or guardians. Information recorded included age, weight, duration of diarrhoea, symptoms of dehydration, vomiting, number and consistency of stools, and non-specific medication received within the previous week. Growth status was expressed separately for males and females by weight-for-age Z-

scores (WAZ).<sup>3</sup> Moderate or severe malnutrition was defined as  $WAZ \leq -2$ . Treatment received from the dispensary was recorded. Data were collected by nurses using a case research form (CRF).

### *Ethical considerations*

Informed consent was obtained from the children's parents or guardians. The study protocol was approved by the Senegalese Health Research National Council.

### *Laboratory methods*

Stool samples were collected in a sterile plastic vial. A wet smear was microscopically examined for intestinal protozoa trophozoites. Stool samples were delivered within 2 hours to the laboratory of the Institut Pasteur for subsequent processing. A portion of stool was concentrated by the merthiolate iodine formaldehyde concentration technique and examined for helminth eggs and protozoa cysts.<sup>4</sup> A rapid chromatographic immune-assay detecting simultaneously rotaviruses and enteric adenoviruses was performed (VIKIA Rota-Adeno, bioMérieux, Marcy l'Etoile, France). Stool specimens were inoculated on to Hektoen Enteric agar (Becton-Dickinson, Heidelberg, Germany) to isolate *Salmonella* and *Shigella* species, and bromocresol purple lactose agar (BCP) (Becton-Dickinson) to isolate *E. coli* and other Gram-negative bacteria. Preston broth medium made in the laboratory was inoculated to enrich *Campylobacter* species and subcultured onto Karmali selective agar (Oxoid, Basingstoke, England).<sup>5</sup> Muller–Kauffmann tetrathionate broth (Bio-Rad, Marnes-la-Coquette, France) was used to enrich *Salmonella* and subcultured onto Rambach agar (Merck, Darmstadt, Germany). Serogrouping and serotyping of *Shigella* isolates were conducted with commercial antisera (Statens Serum Institut, Copenhagen, Denmark and Denka-Seiken, Tokyo, Japan, respectively). *Salmonella* isolates were serotyped according to the Kauffmann–White

scheme using commercial antisera (Statens Serum Institut).<sup>6</sup> Antibiograms were performed by the disc diffusion technique according to the guidelines of the Antibigram Committee of the French Society for Microbiology (CASFM). *E. coli* ATCC 25922 was used as the control strain. *Vibrio cholerae* was not investigated because there were no reported outbreaks during the study duration.

To improve assessment of the real involvement of *Shigella* in diarrhoea, stool samples were stored at -20°C for subsequent testing by PCR assay based on amplifying the invasion plasmid antigen H (*ipaH*) gene, contained in the four *Shigella* species, as well as in entero-invasive *E. coli* (EIEC).<sup>7</sup> *Shigella flexneri* serotype 5 wild-type strain M90T was used as control strain for *ipaH* PCR. Prior isolation of bacterial DNA was performed using the QIAamp DNA Stool Mini Kit (Qiagen, Courtaboeuf, France).

Strains previously identified as *E. coli* using the API20E gallery (bioMérieux, Marcy l'Etoile, France) were screened with a single-test multiplex PCR for the presence of 12 genes to differentiate between the six currently recognised diarrhoeagenic *E. coli* pathotypes.<sup>8</sup> These pathotypes include enteropathogenic *E. coli* (EPEC), atypical EPEC (ATEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC) and entero-aggregative *E. coli* (EAEC). Atypical EPEC (ATEC) strains harbour the LEE (locus of enterocyte effacement) as EPEC strains, but not the EAF (EPEC adherence factor). Simultaneous detection of *E. coli*-specific *uidA* gene was used to confirm biochemical identification of the isolate as *E. coli*, and to serve as PCR control.<sup>8</sup>

### *Statistical analysis*

The study population was described as mean (SD) or median [interquartile range] for continuous variables and as percentages for discrete variables. Relations between clinical variables and pathogens were analysed using the Fisher's Exact test. A test for trend across ordered variables was

used to assess relationships between the different pathogens and patient age. Statistical analysis was performed using the STATA 11 software.

## Results

### *Population*

Between September 2007 and March 2008, 176 children were recruited consecutively to the study (Table 1). Fifty-five per cent were male, and the median age was 13 months [IQ1 8, IQ3 20]. Thirty-six per cent were malnourished ( $Z$ -score  $\leq -2$ ). The median number of stools per day was four [IQ1 3, IQ3 5]. Before attending the dispensary, 20% of children had received oral rehydration salts (ORS), and 23% had received non-specific anti-diarrhoeal agents (e.g. smectite), anti-motility agents (e.g. loperamide), anti-helminthic agents (e.g. albendazole) or medicinal plants (e.g. guava leaves). Temperature was  $\geq 38^{\circ}\text{C}$  in 30% of cases, there were clinical signs of moderate dehydration (two or more signs of the following: restlessness, sunken eyes, thirst and positive skin pinch test) in 59%, vomiting in 61%, and abdominal pain in 42%. None had severe dehydration. Blood was macroscopically seen in 11% of stool samples.

[t]TABLE 1[/t]

### *Pathogens*

At least one of the sought-for pathogens was identified in 105 (60%) children, two pathogens were identified in 25 children, three in two children and four in one child. Bacterial and viral pathogens

were detected in 48 (27%) and 55 (31%) stools, respectively, while parasitic elements were detected in 25 (14%) samples. Mixed bacterial/viral infections were detected in 11 (6%) children.

The pathogens identified in stool samples are shown in Table 2. Rotavirus was the most prevalent pathogen, detected in 46 (27%) stools, 33 (72%) of them in January. The presence of rotavirus was significantly related to vomiting (43% of children with rotavirus had vomiting vs 10% of those without rotavirus,  $P<0.001$ ), absence of blood in stool (0 vs 30%,  $P=0.002$ ), and normal body temperature (32% vs 8%,  $P=0.001$ ), but not to clinical signs of dehydration (27% vs 29%,  $P=0.78$ ). Enteric adenovirus was found in 13 (8%) patients.

### [t]TABLE 2[/t]

*Shigella* was isolated from 21 stools (12%). Sixteen were identified as *Shigella flexneri*, four as *Shigella sonnei* biotype g, and one as *Shigella boydii* serotype 2. Among the 16 isolates of *S. flexneri*, six belonged to the serotype 2a, six to the serotype 1b, three to the serotype 4a, and one to the serotype 3a. Most of the *Shigella* isolates were resistant to cotrimoxazole (overall resistance rate 95%). Rates of resistance of *S. flexneri* strains to amoxicillin, amoxicillin–clavulanic acid, ticarcillin and chloramphenicol were 44%, 44%, 44% and 37%, respectively, whereas all *S. sonnei* strains were susceptible to these antibiotics. All *Shigella* strains were susceptible to third-generation cephalosporins (cefotaxime) and quinolones (nalidixic acid, ciprofloxacin). Non-typhi *Salmonella enterica*, *Campylobacter jejuni* and *P. shigelloides* were isolated from seven (4%), six (3%) and three (2%) stool specimens, respectively. All *Salmonella* strains were resistant to cotrimoxazole but remained susceptible to aminopenicillins, first- and third-generation cephalosporins, chloramphenicol and quinolones.

The *ipaH* gene was detected in all 21 *Shigella* culture-positive specimens and in 30/148 (20%) culture-negative specimens (DNA amplification failed in seven stools). Since recent advances have confirmed that EIEC and *Shigella* should be considered a single pathotype of *E. coli*, 51/169 (30%) samples could be finally considered positive for the entity *Shigella*/EIEC.<sup>9</sup> Presence

of Shigella/EIEC was significantly associated with blood in the stool (84% vs 23%,  $P<0.001$ ) and tended to be associated with fever (40% vs 27%,  $P=0.099$ ).

Diarrhoeagenic *E. coli* were detected in 14 children (8%): EPEC (6 isolates), EAEC (4 isolates), ATEC (2 isolates), ETEC (2 isolates). Neither STEC nor EIEC was detected.

Parasitic agents were detected in 25 (14%) children, namely *Giardia lamblia* (15 patients), *Entamoeba histolytica/Entamoeba dispar* (8), *Ascaris lumbricoides* (4) and *Trichiuris trichiura* (1). One patient harboured three different parasites.

### *Factors associated with pathogens*

Table 3 shows the distribution of pathogens amongst the three age groups, 0–12, 13–24 and 25–60 months, respectively. The prevalence of rotavirus was significantly higher in children aged 0–24 months than in those over 24 months (31% vs 6%,  $P<0.01$ ). Conversely, bacterial pathogens, when considered all together, were significantly less frequent in those aged 0–24 months than in those over 24 months (24% vs 42%,  $P=0.04$ ). This was particularly so for Shigella. Distribution of diarrhoeagenic *E coli* strains was not significantly different between the age groups. The prevalence of parasites increased significantly with age. The distribution of pathogens according to nutritional status is shown in Table 4. Moderate or severe malnutrition was significantly related to detection of bacterial pathogens considered as a whole ( $P=0.04$ ). No significant association was found between rotavirus infection and malnutrition ( $P=0.14$ ), nor between parasitic detection and malnutrition ( $P=0.94$ ).

### [t]TABLES 3 & 4[/t]

### *Therapeutic management*

Among the 140 children for whom treatment prescribed in consultation was documented, 108 (77%) had received antibiotics. Fifty-seven (53%) had received one antimicrobial agent, 38 (35%) two, 9 (8%) three and 4 (4%) up to four different antimicrobial agents. One hundred and six (76%) children received metronidazole for presumed intestinal protozooses, 82 of whom (77%) also received one or more antibiotics. The most frequently prescribed antibiotics among documented patients were ceftriaxone (51%) and gentamicin (11%) by the IM route, and oral amoxicillin/clavulanic acid (26%) and trimethoprim–sulfamethoxazole (16%). Among the 108 children treated with antibiotics, no potential pathogen was found in 39 patients (36%) [in 26 patients (24%) if we consider results for the detection of Shigella/EIEC by PCR]. A virus was detected without any other associated bacterial or parasitic pathogen in 25 cases (23%). ORS were prescribed to only six patients.

## **Discussion**

Since this was not a case–control study, pathogens detected should be considered only as potential pathogens. Nevertheless, it enabled evaluation of the relative importance of different diarrhoeagenic agents in a population of young children living in an urban district of a major West African city.

Rotavirus is a leading cause of severe acute diarrhoea in children under 5 years of age in Africa,<sup>10</sup> and thus, unsurprisingly, rotavirus was the pathogen most commonly found in our population. A peak in frequency, probably an indicator of an epidemic, was observed in January, one of the coolest and driest months in Dakar. This high frequency during the dry season is consistent with previous studies in neighbouring countries.<sup>11,12</sup> Detection of rotavirus was more frequently associated with younger age (<24 months). There is evidence that the incidence of rotavirus infection decreases with age because effective immunity conferred by repeated infections develops gradually.<sup>13</sup> Moreover, protection against rotavirus is conferred primarily by secretory IgA

antibodies located on the surface of the intestinal mucosa. Therefore, maternal antibodies may confer only limited protection against rotavirus during the first months of life. The presence of caliciviruses (i.e. noroviruses and sapoviruses) was not tested in this study. Nevertheless, it is likely that they were involved in some of our cases. Indeed, a recent study in Dakar children hospitalised for acute gastro-enteritis found an 8% prevalence of caliciviruses.<sup>14</sup>

Shigella is a major public health concern in developing countries, particularly in children,<sup>15</sup> and was detected by culture in 21 samples (12%). Its prevalence (identified by conventional culture) is comparable with that found in Dakar in 1993–1994 (11%).<sup>16</sup> If, however, we consider the *ipaH* PCR results, 51 samples were positive for Shigella/EIEC, thus increasing the total percentage of positive stools from 12% to 30%. Shigella could therefore be considered the most frequently recovered pathogen, suggesting that the true incidence of Shigella in childhood diarrhoea in developing countries may be largely underestimated in studies using only conventional culture techniques. *S. flexneri* was the most commonly isolated species in our patients, whereas *S. sonnei* was detected less frequently. In contrast with industrialised countries, the predominance of *S. flexneri* over *S. sonnei* is common in developing countries and has been linked to poor hygiene.<sup>17</sup> The low number of Shigella isolates limits the significance of antimicrobial susceptibility data. However, the study confirmed a recent report showing that resistance of Shigella to first-line antimicrobials (amoxicillin, amoxicillin–clavulanic acid, chloramphenicol, trimethoprim–sulfamethoxazole) is common in Dakar.<sup>18</sup> In contrast, the latter and our study showed that third-generation cephalosporins and quinolones remained effective.

It is well established that diarrhoeagenic *E. coli* pathotypes are a major cause of diarrhoea in Africa, especially in children.<sup>2</sup> Nevertheless, this aetiology is largely unrecognised because few laboratories are able to perform the appropriate identification techniques (i.e. molecular techniques and/or adherence assays). In this study, typical EPEC was the most frequently recovered pathotype (six strains). In addition, two strains of atypical EPEC (ATEC) were detected. EAEC and ETEC pathotypes were also detected (four and two strains, respectively), in contrast with STEC and EIEC.

Campylobacter is a major cause of childhood diarrhoea in developing countries, especially in children under 2 years of age.<sup>19</sup> We found this pathogen in 3% of cases, a result consistent with prevalences reported in previous studies in West Africa.<sup>12,20</sup>

Diarrhoea associated with bacteria, particularly Shigella, affected children over 1 year of age more commonly. This might be explained by the protection conferred by maternal antibodies during the 1st year of life. We found no particular tendency for diarrhoeagenic *E. coli*. The relationship between malnutrition and severity of diarrhoea is well recognised, especially because of the immune system deficiency caused by inadequate intake of micronutrients such as zinc.<sup>21</sup> In this study, the detection of bacterial pathogens was significantly associated with malnutrition but not with the detection of rotavirus and parasites.

Parasitic agents were mainly protozoa (*Giardia lamblia*, *E. histolytica*/*E. dispar*), while helminth eggs were rarely detected. This low prevalence of helminthiasis may be owing to the young age of the children. A study in Senegal in 2002 showed that the rate of helminth infestation was highest in children between 11 and 15 years of age.<sup>22</sup> The prevalence of parasites (both protozoa and helminths) in this study also tended to increase with age. Another reason for this low prevalence of helminths in our population might be that a number of children had received anti-helminthics before coming for consultation. It is noteworthy that we did not investigate for *Cryptosporidium* species in our study. This pathogen was significantly associated with childhood diarrhoea in West Africa.<sup>23</sup>

The majority of children presented with signs of mild or moderate dehydration. No cases of severe dehydration were recorded, probably because they were directly hospitalised in paediatric wards.

Of those children with documented antibiotic treatment prescribed in consultation, a virus (rotavirus or adenovirus) was detected without any other associated pathogen in 23% of cases. Moreover, no pathogen was found in 36% of children. Although this rate could be reduced to 24% when the results of PCR *ipaH* for detecting Shigella are considered, antibiotic treatment was

unnecessary in approximately half of the cases. This over-use of antibiotics is of concern because it promotes the emergence of antibiotic resistance. It also represents a significant cost to families.

This report underscores that accurate evaluation of pathogens in childhood diarrhoea in developing countries requires the integration of advanced methods that complement existing tools. Moreover, it highlights the need for primary health care facilities to perform certain simple tests that assess the appropriateness of antibiotic treatment. Microscopic examination of stools can be done easily if staff are trained in basic parasitology. At the same time, however, simple, reliable and inexpensive diagnostic tools for detecting other pathogens, especially bacteria, are needed.

Immunochromatographic tests (dipsticks) are being developed for *S. flexneri* 2a and *S. dysenteriae* 1.<sup>24,25</sup> These tools, although promising, require further evaluation in routine practice. They also need to be developed for other pathogens, particularly other species of *Shigella* and diarrhoeagenic *E. coli*.

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Table 1 Characteristics of the 176 children enrolled in the study

Characteristics	<i>n</i> (%)
Male	97 (55)
Age, mths*	13 [8, 20]
Z-score*	-1.3 [-2.6, -0.4]
≤-2 (19 missing)	56 (36)
Duration of diarrhoea*, days	3 [3, 6]
Number of stools per day*	4 [3, 5]
Received previous ORS <sup>†</sup> therapy (13 missing)	33 (20)
Received previous non-specific medication (1 missing)	41 (23)
Received one or more antibiotics in consultation (36 missing)	108 (77)
Body temperature ≥38°C (8 missing)	50 (30)
Symptoms of moderate dehydration (14 missing)	96 (59)
Vomiting (19 missing)	96 (61)
Abdominal pain (35 missing)	59 (42)
Blood in stool	20 (11)

\* Median [IQ1, IQ3]; <sup>†</sup> oral rehydration salts.

Table 2 Enteric pathogens detected in 176 children aged <5 years attending Saint-Martin Dispensary

Cause	Pathogen	<i>n</i> (%)
Bacteria	Diarrhoeagenic <i>E. coli</i>	14 (8)
	EPEC	6
	EAEC	4
	ATEC	2
	ETEC	2
	<i>Shigella</i> spp.	21 (12)
	<i>S. flexneri</i>	16
	<i>S. sonnei</i>	4
	<i>S. boydii</i>	1
	<i>Salmonella</i> spp.	7 (4)
	<i>Campylobacter jejuni</i>	6 (3)
	<i>Plesiomonas shigelloides</i>	3 (2)
Viruses*	Rotavirus	46 (27)
	Adenovirus	13 (8)
Parasites	<i>Giardia lamblia</i>	15 (9)
	<i>E. histolytica/E. dispar</i>	8 (5)
	<i>Ascaris lumbricoides</i>	4 (2)
	<i>Trichiuris trichiura</i>	1 (<1)

\* Data missing for five patients.

Table 3 Distribution of identified pathogens according to age

Organism	Age group, mths	No. (%) of children infected	P-value
Rotavirus ( <i>n</i> =46)	0–12	29/81 (36)	<0.01
	13–24	15/59 (25)	
	25–60	2/31 (6)	
Bacteria* (all species) ( <i>n</i> =48)	0–12	18/85 (21)	0.03
	13–24	17/60 (28)	
	25–60	13/31 (42)	
<i>Shigella</i> spp* ( <i>n</i> =21)	0–12	4/85 (5)	<0.01
	13–24	10/60 (17)	
	25–60	7/31 (23)	
<i>Shigella</i> /EIEC <sup>†</sup> ( <i>n</i> =51)	0–12	17/80 (21)	<0.01
	13–24	20/58 (34)	
	25–60	14/31 (45)	
Diarrhoeagenic <i>E. coli</i> <sup>‡</sup> ( <i>n</i> =14)	0–12	9/85 (11)	0.17
	13–24	4/60 (7)	
	25–60	1/31 (3)	
Parasites ( <i>n</i> =25)	0–12	7/85 (8)	0.01
	13–24	10/60 (17)	
	25–60	8/31 (26)	

\* Identified by culture; <sup>†</sup> determined by *ipaH* PCR; <sup>‡</sup> selected by culture and confirmed by multiplex PCR.

Table 4 Distribution of identified pathogens according to nutritional status ( $n=157$ , 19 missing values)

Organism	Normal nutritional status $n=101$ (%)	Malnourished $n=56$ (%)	<i>P</i> -value
Rotavirus ( $n=42$ )	31/98 (32)	11/54 (20)	0.14
Bacteria* (all species) ( $n=41$ )	21/101 (21)	20/56 (36)	0.04
<i>Shigella</i> spp* ( $n=17$ )	8/101 (8)	9/56 (16)	0.12
<i>Shigella</i> /EIEC <sup>†</sup> ( $n=42$ )	24/97 (25)	18/53 (34)	0.23
Diarrhoeagenic <i>E. coli</i> <sup>‡</sup> ( $n=13$ )	5/101 (5)	8/56 (14)	0.07
Parasites ( $n=22$ )	14/101 (14)	8/56 (14)	0.94

\* Identified by culture; <sup>†</sup> determined by *ipaH* PCR; <sup>‡</sup> selected by culture and confirmed by multiplex PCR. Normal nutritional status was defined as weight-for-age Z-score  $>-2$ , moderate or severe malnutrition as weight-for-age Z-score  $\leq -2$ .